BIOMONITORS: PRACTICE AND CONCEPTS

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ABSTRACT

The usefulness of organisms as biomonitors of environmental quality has been demonstrated repeatedly during episodes of acute poisoning. Management goals now largely seek to avoid or mitigate these occurrences, concomitant with introduction of chemicals to the environment. Biomonitors have largely been adopted as sentinel organisms to warn of excess bioavailability of xenobiotics. Uptake and retention processes in any organisms, however, are only semi-passive, so that metabolic processes may influence both concentration ranges over which accumulation occurs, and mechanisms controlling it. Furthermore, the relationship between route and extent of accumulation is usually not known and cannot necessarily be inferred. Recently-proposed models use knowledge of a substance's physicochemical properties to predict its disposition in environmental compartments, e. g. water, air, sediments and biota, based on related thermodynamic concepts of equilibrium partitioning or fugacity. It is not clear, however, that prediction of the behavior of chemicals not near equilibrium can adequately be made, at least for management purposes. These new concepts form the basis of a need for better understanding of physiological and biochemical attributes of biomonitor species and the significance of physico-chemical behavior so that both the quality and limitations of entries in data bases created during monitoring programs is clearly understood.

INTRODUCTION

The foundation of institutional concern for the environment is composed of examples of acutely deleterious effects of environmental contamination on animal (1-5) and human (6) populations. The affected groups inadvertently served as sentinels which initiated subsequent chemical investigations to determine the identity and mode(s) of action of the causative agent. In the last 30 years during which these examples occurred, chemical analysis has been refined to allow determinations of elements and molecular structures with increasing accuracy, sensitivity and efficiency, making routine environmental surveillance by chemical means possible. Concomitantly, schemes using biomonitors have also been implemented, the best example of which is the "Musselwatch" (7-9). Chemical analysis methods are highly specific but in many cases lack generality. The primary need is to identify those substances or their chemical speciation products which accrue to a harmful level in living organisms, including humans. Biomonitors address this need directly if they accumulate the material by a generalized route. Biomonitoring may operate more effectively and efficiently in the environment than other strategies. The adoption of biomonitors, however, is based upon an implicit set of assumptions which translate into limitations on the interpretation or application of data. These predictable limitations are the subject of this paper.

Biomonitors respond to environmental conditions in unique ways and should not be viewed as living sponges passively accumulating xenobiotics. Dead organisms accrue chemicals very differently in pattern and extent than living ones. In the broadest sense, bioaccumulation can be explained as the ratio of rates of accumulation over elimination (10,11). Both processes are influenced by physiological functions controlling contact with the environment, e.g. flow of water over respiratory surfaces, transfer of chemicals from the site of entry via circulating body fluids, enzymatic modification and excretory processes. Because exposure to toxic chemicals, the subjects of the monitoring exercises, may cause mortality, the potential application of biomonitors is limited. Their most successful use has been for conditions of chronic, sublethal exposure. This is perhaps the most significant difference between organisms and chemical methods. If the species chosen as a biomonitor is a surrogate for another, perhaps less experimentally tractable one, it must not be significantly more sensitive to toxic effects of the chemical(s) of interest. A potentially narrower range of concentrations over which biomonitors provide acceptable performance is perhaps the most significant difference between them and more traditional sampling protocols.

A major function of biomonitors is to show that xenobiotics can be transferred from one environmental compartment, usually but not always nonliving, into the living organisms. Species which specifically and preferentially concentrate materials of interest are the ones of choice. One species of sabellid polychaetes has been documented to accumulate vanadium and titanium (12). Many ascidian species notably concentrate vanadium from seawater by specific metabolic pathways (13). In

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cannot be increased in response to increased concentrations in food unless specific inducible enzymatic mechanisms, the mixed function oxidase system (MFO) (29,30), are operative. In aquatic food chains, the best example of food chain accumulation is that of mercury. Highest mercury burdens are found in marlin and sailfish (31), top predators in the food web, and maintained in these fish by binding to metallothioneins.

FATE WITHIN THE ORGANISM

After chemicals have been taken up, mechanisms controlling their distribution in tissues and elimination become important in bioaccumulation processes. Partitioning of hydrophobic chemicals is dominated by their physico-chemical properties (32-34). Thus, highest potential burdens will vary with the chemical under consideration and depend upon the ratio of chemical potentials in the aqueous and hydrophobic compartment. Mass accumulation within a given tissue is also a function of the proportion of lipid or other biochemicals serving as a hydrophobic refuge (35). Nevertheless, enzymatic and physiological processes may play a significant role either by chemically modifying the compounds to enhance water solubility (36) or by modifying processes which favor the compound's release (37). For organic compounds, the external concentration may markedly influence the ability of endogenous factors to effectively mediate release of xenobiotics (38). The case of metals and their binding to metallothioneins, mentioned above, is an example where retention by inducible, but metabolically inactive biochemical moieties markedly influences accumulation. Accumulation and retention of metals continues as long as metallothionein synthesis does. When exposure to metals ceases, elimination of excess burdens of many, but not all, occurs. The mechanisms governing the elimination process are not well understood in all cases (26). The message here is that elimination of substances whose uptake and retention is not mediated by specific processes is usually more rapid and more predictable regardless of the original entry route. Where specific mechanisms for modifying or sequestering xenobiotics occur, elimination may be slower and less predictable.

Time is perhaps the most underappreciated element of biomonitoring strategies. Organisms may grow, proceed through daily, seasonal or annual cycles affecting their efficacy as biomonitors. The effects of these cycles may be very subtle. Blue crabs, Callinectes sapidus, accumulated kepone in the ovaries and then lost it to the eggs (and resultant larvae) when they were deposited on the pleopods (39). Gravid mussels, Mytilus edulis dramatically accumulated tributyltin in their gonads (18,38). When organisms are simple selected from various sites for analysis of tissue burdens of chemicals of interest, biological indices such as sex, reproductive condition or season are highly recommended as correlation covariables in statistical analyses.

The duration of exposure, actual or potential appears to be very important in bioaccumulation. Burdens of mercury in marlin and sailfish increase as a function of size of the animal (31). Presumably, larger fish are older so that the increased tissue mercury concentrations reflect continuing accrual throughout life. Organic compounds with low water solubility ($K_{ow} > 10^5$) may also display this behavior. Tributyltin did not appear to reach a steady state in marine mussels, Mytilus edulis during 45 days exposure (18) or fish during a similar exposure period (40). There is a subset of xenobiotics, whose size is presently unknown, which take days, months or years, perhaps longer than the usual life span of many organisms, to reach steady state. In these cases, knowledge of the duration of potential exposure is essential for data interpretation.

PROSPECTUS

Biomonitors have an obvious and necessary place in environmental studies. Their use now rests upon a solid foundation of empiricism and increasingly, theory. There is still, however, need for critically devised laboratory experiments to better understand inducible biochemical and physiological mechanisms of xenobiotic modification and release which are important at environmental concentrations of compounds of interest. Such studies are particularly important for, but not limited to, novel compounds such as those containing tin, arsenic, selenium and organometallic derivatives of these elements.

It is no longer adequate for chemical analysis of tissues or cells of biomonitors to show that a parent compound is not present. Conjugates and some metabolites may pose identical or greater risks. Continued development of and reliance upon state of the art analytical chemical techniques will remain essential as a concomitant to the use of biomonitors.

Numerous suitable biomonitor species exist. Many are underutilized and most remain to be described and used. Conformity in the adoption of biomonitor species is no virtue, but the goal should always be a phenomenological approach rather than toward the specific example. Microorganisms, in particular, have been overlooked as potential biomonitors, an odd omission given their numerical and function dominance in nearly all ecological systems.

The use of biomonitors in the marine environment is no longer limited simply to xenobiotic surveillance. Recent studies have reported attempts to correlate neoplastic lesions in fish with the presence of chemicals in their environment and tissue (41,42). The relationship between the concentration of a substance in the environment a chemist measures, the dose an organism accumulates, and the response it produces measurable by a biologist is perhaps one of the most challenging subjects of interdisciplinary investigation. Benefits will doubtless extend beyond the immediate problems engendering the

other organisms, metals such as copper (14) and mercury (15) may be sequestered into specific protein classes which can be specifically isolated and their metal content quantified. Use of mussels in surveillance of the marine environment for hydrocarbons illustrates a completely different strategy. There are no specific metabolic pathways for uptake or retention, but degradation/elimination processes are also limited so that accumulated materials remain in tissues for much longer than would otherwise occur (16,17). Observation of accumulation of an element or compound preceeded an understanding of mechanisms in nearly all cases of organisms subsequently adopted as biomonitors. However, as novel anthropogenic compounds become of interest, one can apply generalizations in the selection of species as biomonitors (18).

ENVIRONMENT AND NICHE

Biomonitors sample their niche. In some cases, the niche may be more extensive and complex than the physical environment. For instance, an infaunal organism such as the tube worm Chaetopterus which filters water by drawing it through its buried tube would likely accumulate tissue burdens reflecting those in the water rather than in the sediment around it. Ecological knowledge about the niche is essential to determine how well it overlaps with the environmental compartment of interest so that species can be selected to adequately portray the environmental compartment of most significance. Conversely, differences in xenobiotic bioavailability to several ecologically distinct species may provide clues to its movement through the environment.

In aquatic environments, the three primary compartments, in addition to organisms, are the atmosphere, water and sediments (19,20). The atmosphere may be an important route of xenobiotics into aquatic environments but unless chemicals are volatile or possess highly favorable Henry's law constants, it is rarely a sink. Accumulation from water is perhaps the best understood process. Since many toxics enter the water as effluents or land runoff, concern over dissolved compounds is appropriate. In addition, physico-chemical properties of the dissolved materials, to be discussed below, dominate accumulation mechanistically. Sediments are the primary sink in the environment for most highly hydrophobic materials and reactive chemical species such as metal ions. Depending upon the substance, both organic or inorganic sediment components may be important in xenobiotic capture.

Strict adherence to compartment concepts is necessary for mathematical modelling but should not be dogmatically accepted. The models are based upon equilibrium concepts. Kinetic factors are more difficult to predict or measure but play a very important role. At best, biomonitors may only represent one small segment in a largely directional but nonlinear and unscheduled transfer. Resuspension of sediment by water movement and bioturbation make sediment bound xenobiotics bioavailable longer than would otherwise be the case. Equilibrium models do not consider the case where the movement of xenobiotics cycle or appear to cycle between compartment. This aspect is particularly important for very persistent chemicals or elements. A biomonitor's role in such a cycle must be clearly understood for adequate data interpretation.

Complexity in the compartmental structure of the environment is the basis for functional and temporal patterns of bioaccumulation. For instance, DiSalvo et. al (21) found in mussel tissues that hydrocarbon profiles following an oil spill more closely resembled those in sediments than those in the water. Several explanations for this are possible, but it seems most likely that mussels obtained hydrocarbons from the sediment, even though these filter feeders were not in direct contact with it. There has been some attention lately to separating the roles of bound and dissolved metal ions in bioaccumulation processes (22,23). Accumulation of metal ions illustrates temporal dependence. Toxicity of metals is dependent upon the stability of metal-ligand complexes (24,25). The solubility products between most metals and biologically produced ligands are between 10^{-10} and 10^{-25} (26,27), reflecting strong but not exceptional binding. In sediments, reaction with S^{2-} dominates metal binding chemistry because stability constants are less than 10^{-40} (28). Clearly, in a system at equilibrium, sediments ought to contain all metals as sulfides. These metals, both trace nutrients and toxics above threshold concentrations, occur ubiquitously in living tissues because ecological compartmentalization is not near physico-chemical equilibrium. These observations suggest that species chosen as biomonitors must appropriately sample the compartment of interest over an appropriate time scale. In practice, they will probably sample both water and sediment. Temporally, they will also act as integrators. A spike of heightened exposure, essentially even a brief excursion from steady state, may be retained in tissues well past the time the crisis has past and ambient water levels have returned to background. Coincident independent sampling is essential to determine the return toward initial conditions if the input of xenobiotics has occurred during a spill or other crisis situation.

Accumulation of xenobiotics via the food chain is another route which influences the appropriateness of an organism as a biomonitor and weighs heavily in the interpretation of data. When feeding is selective e.g. deposit feeders, predators or highly sophisticated, e.g. filter feeding bivalves, the importance of food chain accumulation should be scrutinized carefully. Accumulation from food is likely to markedly increase both rates and levels of accumulation since there is a direct mass transfer of material in digested food into the organism (18). Consumption of contaminated food markedly displaces the steady state between accumulation and release of xenobiotics primarily because elimination mechanisms

effort. Developments in this field should continue to be among the most valuable products of science in the latter part of the twentieth century.

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REFERENCES

1. Carson, R. Silent Spring. Riverside Press, Cambridge, Massachusetts, 1962.
2. Koeman, J.H. Chlorinated biphenyls in fish, mussels and birds from the River Rhine and the Netherlands coastal area. Nature 1969 221, 1121-1128.
3. Kazantsis, G. The poison chain for mercury in the environment. International Journal of Environmental Studies 1971 1, 301-306.
4. Alzieu, C., Y. Thibaud, M. Heral and B. Boutier. Evaluation des risques dus a l'emploi des peintures anti-salissures dans les zones conchylicoles. Revues Travails Institute des Peches Maritimes 1980. 44, 301-343.
5. Waldock, M.J. & J. Thain. Shell-thickening in Crassostrea gigas. Organotin antifouling or sediment induced? Marine Pollution Bulletin 1983 14, 411-415.
6. Thayer, J.S. Organometallic Compounds and Living Organisms. Academic Press, Orlando, Florida, 1984.
7. Goldberg, E.D., V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider & E. Gamble. Mussel Watch. Environmental Conservation, 1978 5, 101-124.
8. Farrington, J.W., E.D. Goldberg, R.W. Risebrough, J.H. Martin & V.T. Bowen. U.S. "Mussel watch" 1976-1978: An overview of trace metal, DDE, PCB, hydrocarbon and artificial radionuclide data. Environmental Science & Technology 1983, 17, 490-496.
9. Martin, M. State Mussel Watch: Toxics surveillance in California. Marine Pollution Bulletin 1985 16, 140-146.
10. Neely, W.B. Estimating rate constants for the uptake and clearance of chemicals by fish. Environmental Science & Technology 1979 13, 1506-1510.
11. Spacie, A. & J.L. Hamelink. Alternative models for describing the bioconcentration of organics in fish. Environmental Toxicology and Chemistry 1982, 1, 309-320.
12. Popham, J.D. & J.M. D'Auria. A new sentinel organism for vanadium and titanium. Marine Pollution Bulletin 1982 13, 25-27.
13. Stoecker, D. Relationships between chemical defense and ecology in benthic ascidians. Marine Ecology Progress Series 1980 3, 257-265.
14. Sanders, B.M. & K.D. Jenkins. Relationships between free cupric ion concentrations in seawater and copper metabolism and growth in crab larvae. Biological Bulletin 1984 167, 704-712.
15. Roesijadi, G. Uptake and incorporation of mercury into mercury binding proteins of gills of Mytilus edulis as a function of time. Marine Biology 1982 66, 151-157.
16. Lee, R.F., R. Sauerheber & A.A. Benson. Petroleum hydrocarbons: uptake and discharge by the marine mussel, Mytilus edulis. Science 1972 177, 344-346.
17. Krieger, R.I., S.J. Gee, L.O. Lim, J.H. Ross, A. Wilson, C. Alpers and S.R. Wellings. Disposition of toxic substances in mussels (Mytilus californianus): Preliminary metabolic and histologic studies. In Pesticide and Xenobiotic Metabolism in Aquatic Organisms ed. by M.A.Q. Khan, J.J. Lech & J.J. Menn. American Chemical Society, Washington, D.C. pp. 259-257. 1979.
18. Laughlin, R.B., Jr., W. French & H.E. Guard. Accumulation of bis(tributyltin) oxide by the marine mussel Mytilus edulis. Environmental Science & Technology (In press).
19. Mackay, D. Finding fugacity feasible. Environmental Science & Technology. 1979 13, 1218-1223.
20. Mackay, D. & S. Peterson. Fugacity revisited. Environmental Science & Technology 1982. 16, 654A-660A.
21. DiSalvo, L.H., H.E. Guard & L. Hunter. Tissue hydrocarbon burden of mussels as potential monitor of environmental hydrocarbon insult. Environmental Science & Technology 1975 9, 247-251.
22. Luoma, S.N., D. Cain & C. Johansson. Temporal fluctuations of silver, copper and zinc in the bivalve Macoma balthica at five stations in south San Francisco Bay. Hydrobiologia 1985 129, 109-120.
23. Harvey, R.W. & S.N. Luoma. Separation of solute and particulate vectors of heavy metal uptake in controlled suspension-feeding experiments with Macoma balthica. Hydrobiologia 1985 121, 97-102.
24. Irving, H. & R.J.P. Williams. Order of stability of metal complexes. Nature 1948 162, 746-747.
25. Shaw, W.H.R. Cation toxicity and the stability of transition metal complexes. Nature 1961. 192, 746-747.
26. Simkiss, K. & M. Taylor. Cellular mechanisms of metal ion detoxification and some new indices of pollution. Aquatic Toxicology 1981. 1, 279-290.
27. Simkiss, K., M. Taylor & A.Z. Mason. Metal detoxification and bioaccumulation in molluscs. Marine Biology Letters 1982, 3, 187-201.
28. Stumm, W. & J.J. Morgan. Aquatic Chemistry 1981. Wiley & Sons, New York.
29. Lee, R.F. Mixed function oxygenases (MFO) in marine invertebrates. Marine Biology Letters 1981 2, 87-105.
30. Varanasi, U. & D.C. Malins. Metabolism of petroleum hydrocarbons: Accumulation and biotransformation in marine organisms. In: Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms. Volume II. Biological Effects. Academic Press, New York 1977 pp. 175-270.

31. Barber, R.T. & P.J. Whaling. Mercury in Marlin and Sailfish. Marine Pollution Bulletin 1983 14, 395-396.
32. Mackay, D. Correlation of bioconcentration factors. Environmental Science & Technology 1982 16, 274-278.
33. Geyer, H., P. Sheenan, D. Kotzias, D. Freitag, & F. Korte. Prediction of ecotoxicological behavior of chemicals: Relationship between physico-chemical properties and bioaccumulation of organic chemicals in the mussel *Mytilus edulis*. Chemosphere 1982 11, 1121-1134.
34. Veith, G.D., K.J. Macek, S.R. Petrocelli & J. Carroll. An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. In: Aquatic Toxicology, ASTM STP 707 ed. by J.G. Eaton, P.R. Parrish & A.C. Hendricks. American Society for Testing and Materials, Philadelphia, Pa. 1980 pp. 116-129.
35. Mackay, D. & A.I. Hughes. Three parameter equation describing the uptake of organic compounds by fish. Environmental Science & Technology 1984 18, 439-444.
36. Livingstone, D.R. Responses of the detoxification/toxication enzyme systems of molluscs to organic pollutants and xenobiotics. Marine Pollution Bulletin 1985 16, 158-164.
37. Widdows, J., S.L. Moore, K.R. Clarke & P. Donkin. Uptake, tissue distribution and elimination of [1-^{14}C]naphthalene in the mussel, *Mytilus edulis*. Marine Biology 1983 76, 109-114.
38. Laughlin, R.B., Jr. & W. French. Concentration dependence of bis(tributyltin) accumulation in the marine mussel, *Mytilus edulis* (submitted to Marine Pollution Bulletin).
39. Roberts, M.H. Jr. & A.T. Leggett, Jr. Egg extrusion as a kepone-clearance route in the blue crab, *Callinectes sapidus*. Estuaries 1980 3, 192-199.
40. Ward, G.S., G.C. Cramm, P.R. Parrish, H. Trachman & A. Slesinger. Bioaccumulation and chronic toxicity of bis(tributyltin) oxide (TBTO): Tests with a saltwater fish. In: Aquatic Toxicology and Hazard Assessment: Fourth Conference ASTM STP 737, ed. by D.R. Branson and K.L. Dickson. American Society for Testing and Materials, Philadelphia, Pa. 1981 pp. 183-200.
41. Ginn, T.C. & R.C. Barrick. Bioaccumulation of toxic substances in English sole (*Parophrys vetulus*) from Commencement Bay (Puget Sound, Washington). Paper presented to the 6th International Ocean Disposal Symposium, Asilomar, California, 21-25 April, 1986.
42. Becker, D.S. & T.C. Ginn. Hepatic lesions in English sole (*Parophrys velutus*) from contaminated areas of Commencement Bay. Paper presented to the 6th International Ocean Disposal Symposium, Asilomar, California, 21-25 April, 1986.

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